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ARTICLE

Histology of genital tract and breast tissue after long-term testosterone administration in a female-to-male transsexual population

Michaël Grynberg [a,b,c,*], Renato Fanchin [a,b,c], Geneviève Dubost [d], Jean-Claude Colau [e], Catherine Brémont-Weil [f], René Frydman [a,b,c], Jean-Marc Ayoubi [e]

[a] *AP-HP, Service de Gynécologie-Obstétrique et Médecine de la Reproduction, Hôpital Antoine Béclère, 157, rue de la Porte de Trivaux, Clamart F-92141, France;* [b] *University Paris-Sud, Clamart F-92140, France;* [c] *INSERM, U782, Clamart F-92140, France;* [d] *Department of Pathology, Hôpital Foch, Suresnes, France;* [e] *Department of Obstetrics and Gynecology, Hôpital Foch, Suresnes, France;* [f] *Department of Endocrinology, Hôpital Cochin, Assistance Publique-Hôpitaux de Paris, Paris, France*

[*] Corresponding author. *E-mail address:* michael.grynberg@abc.aphp.fr (M Grynberg).

Michaël Grynberg is a gynaecologist specializing in reproductive medicine. After spending 6 months as a visiting fellow in the Department of Reproductive Medicine at Cornell University, he returned to work in France and is currently undertaking a fellowship in the Division of Reproductive Medicine at the Hôpital Antoine Béclère, Clamart, France. His scientific interests centre on the identification of clinical markers of ovarian function, the role of androgens during the folliculogenesis and the physiopathology of PCOS. In addition, he is currently a PhD student in the University Paris-Sud 11. His thesis focuses on anti-Müllerian hormone regulation in the human ovary.

Abstract Growing evidence indicates that androgens play a positive role in follicle proliferation and growth. Hence, many authors have assumed that androgen supplementation in women with poor ovarian reserve might improve the number of antral follicles available for ovarian stimulation. As androgen administration may become more frequently used in reproductive medicine, this study aimed at describing the histological changes observed in the genital tract and the breast of female-to-male (FTM) transsexuals. A pathological analysis of the genital tract of 112 FTM subjects who were given androgen for at least 6 months before hystero-salpingo-oophorectomy was performed. In addition, 100 bilateral mastectomies were performed, allowing a study of the breast tissue. Mean ovarian volume was increased, with histological characteristics of polycystic ovaries (PCO), defined as >12 antral follicles per ovary, observed in 89 patients (79.5%). Endometrial atrophy was observed in 45%. Breast examination revealed marked reduction of glandular tissue and increase of fibrous connective tissue in 93%, without atypical hyperplasia or carcinoma. The present data confirms and expands the putative associations between long-term androgen administration and abnormalities in ovarian architecture with macroscopic and microscopic characteristics of PCO, increased risk of endometrial atrophy and fibrotic breast tissue with marked glandular reduction. RBMOnline

KEYWORDS: androgens, breast, genital tract, ovary, polycystic, transsexual

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Introduction

Gender identity disorder is a relatively rare condition of atypical gender development in which there is a psychological perception of self as masculine or feminine which is incongruent with one's phenotype. It is described in the International Classification of Diseases 10th revision (ICD-10; World Health Organization, 1992) and the Diagnostic and Statistical Manual of Mental Disorders — Text Revision IV (DSM-IV-TR; American Psychiatric Association, 1994). Affected patients usually need medical interventions to facilitate their transition to live in accordance with the identified gender rather than with the phenotype. Even though the ICD-10 and DSM-IV-TR descriptions vary slightly, each provides a reliably sound and valid template for diagnosis. Gender identity disorder has a reported incidence worldwide of up to one in 120,000 for female-to-male (FTM) transsexuals (Bakker et al., 2002) and the main tenet of its medical management is long-term hormonal treatment (The Harry Benjamin International Gender Dysphoria Association standards of care for gender identity disorders, 6th version, 2001; Walker et al., 1985). In fact, the acquisition of the secondary sex characteristics of the other gender, which is fundamental to sex reassignment, is contingent on sex steroid therapy. The goal of treatment of the FTM transsexual is to induce virilization, male-pattern body hair growth and physical contours, together with cessation of menstruation. Testosterone preparations are usually used to accomplish these goals. Androgens are usually administered for at least 6 months before the reassignment surgery and continued thereafter. Surgery includes hysterectomy, bilateral salpingo-oophorectomies and mastectomy. FTM transsexual patients represent an excellent model to assess the histological long-term effects of androgens on many tissues, especially on the female genital tract and the breast.

Growing evidence indicates that androgens are involved in the process of folliculogenesis. Indeed, both experimental and clinical data provide good evidence for a critical role of androgens in the control of follicular development and a positive effect on follicle proliferation and growth. Based on these data, many authors have assumed that androgen supplementation in women with poor ovarian reserve might improve the number of small antral follicles available for ovarian stimulation, as well as improve the follicular sensitivity to FSH. However, as the folliculogenesis process lasts several months, a long-term androgen therapy should be used to reach this issue.

Hence, while androgen administration might take a growing place in reproductive medicine, the aim of this study was to describe the histological changes observed in the genital tract of FTM transsexuals after long-term androgen therapy.

Materials and methods

Population

This study retrospectively reviewed the histological data obtained from examining the ovaries, the endometrium and the breast tissue from 112 women, 21—53 years of age, with transsexualism, diagnosed according to the DSM IV criteria (American Psychiatric Association, 1994), who underwent hysterectomy, bilateral salpingo-oophorectomies and mastectomy from January 2000 to December 2006. Prior to surgery, all patients had received androgen therapy as testosterone enantate (Androtardyl; Schering SA, France) 250 mg intramuscularly every 4 weeks for at least 6 months.

Patient characteristics

At the time of sex reassignment surgery, the overall mean age of the 112 patients was 28.9 ± 0.9 years. Their mean body mass index was 25.4 ± 0.2 kg/m^2. Before androgen treatment, 98 had regular cycles (88%). Clitoridomegaly was not found before therapy in any subject. No patient had had ovariectomy before sex reassignment surgery, but they had received androgen treatment for 2—9 years (3.7 ± 0.6 years). During androgen therapy, all the patients became completely amenorrhoeic and masculinized (Ferriman—Gallwey score > 25).

Histopathological examination

Pathological examination was simultaneously performed by two observers. Both ovaries were measured longitudinally, transversely and antero-posteriorly, allowing for calculation of the ovarian volume. Histological sections were made longitudinally throughout the largest diameter of the ovaries. Paraffin sections were stained with haematoxylin and azophloxin (H and A) and Masson's trichrome stain. Histopathological criteria for polycystic ovaries (PCO) were the same as those described elsewhere (Futterweit and Deligdisch, 1986; Hughesdon, 1982; Pache et al., 1991) and included collagenization of the outer cortex, stromal hyperplasia, luteinization of stroma cells and multifollicular ovaries in various stages of growth and atresia. In this latter case, healthy antral follicles were considered those bordered with two or more layers of granulosa cells, cystic follicles were those with partly collagenized wall, and atretic follicles were those that had lost their shape and their granulosa cell content. The number of healthy antral, cystic and atretic follicles was considered consistent with PCO when more than 12 per ovary were found.

For endometrial histology, glandular hyperplasia with cysts was defined as proliferating glands lined with tall stratified columnar epithelium with a high nucleo-cytoplasmic ratio and with a large number of mitotic figures, and cystically dilated glands. An atrophic endometrium consisted of tubular glands lined by cuboidal epithelium with low nucleo-cytoplasmic ratio, no proliferative or secretory activity and separated by a compact stroma. The diagnosis of adenomyosis, characterized by the presence of heterotopic endometrial glands and stroma in the myometrium with adjacent smooth muscle hyperplasia, was made when glandular extension below the endometrial—myometrial interface was >2.5 mm (Uduwela et al., 2000). The staining procedures for endometrium, cervix, fallopian tubes and breast analysis were as follow: haematoxylin eosine (H&E) and toluidine; H&E and PAS; H&E; and H&E, respectively.

For breast histology, sections from mastectomy specimens consisted of one or more sections per breast (2—10

per patient) stained by H and E. Pathological examination focused on looking for intraductal hyperplasia and/or carcinoma.

Statistical analysis

The measure of central tendency used was the mean and the measure of variability was the standard error. Comparisons between ovarian volumes in patients with or without PCO characteristics on histological analysis were performed using Mann–Whitney *U*-tests. Relationship between two continuous variables was assessed by correlation when they were independent from each other. The Spearman's test was used to determine whether coefficients of correlation (r) were significantly different from zero. A P-value <0.05 was considered statistically significant.

Results

Ovarian histology

Ninety-nine out of the 224 ovaries appeared macroscopically enlarged. Mean ovarian right and left volumes were 10.9 ± 0.68 ml, and 10.1 ± 0.66 ml, respectively. The mean number of antral follicles, including healthy antral, cystic and atretic follicles was at 16.5 ± 0.62 per ovary. An example of a section through an ovary of a FTM transsexual showing multiple cystic atretic follicles is shown in **Figure 1**. The ovarian stroma showed hyperplasia in all patients. Considering, these data, histological PCO aspects were observed in 89 patients (79.5%), who displayed more than 12 antral follicles per ovary. Ovarian volumes appeared significantly larger in women with PCO aspect on histological analysis, as compared with those with non-polycystic ovaries (right ovary (RO): $z = -2.8$; $P < 0.006$; left ovary (LO): $z = -3.02$; $P < 0.03$). In addition, mean ovarian volume, (RO + LO)/2, was strongly correlated with the number of antral follicles ($r = 0.67$; $P < 0.0001$).

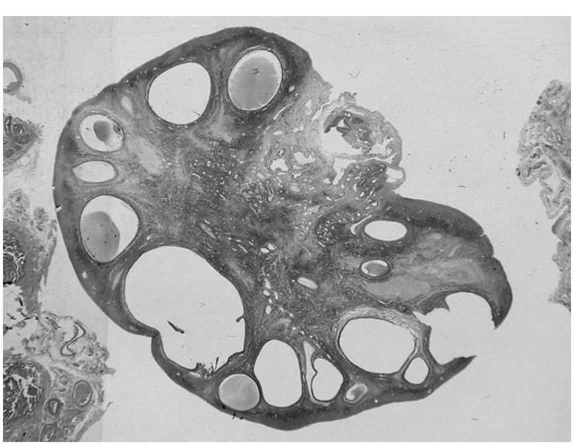

Figure 1 Section through ovary of female-to-male transsexual showing multiple cystic atretic follicles (haematoxylin and eosin staining, 4× magnification).

Endometrium and myometrium histology

Mean uterine length was 7.89 ± 0.15 cm. Two distinct patterns could be observed, proliferative and atrophic endometrium. Proliferative endometrium was noted in 54 cases. Atrophic endometrium was observed in 50 patients, displaying small and tubular glands with stromal condensation. Endometrial polyps were present in four cases, while endometrial hyperplasia, without cellular atypia was described in eight cases. Atypical endometrial hyperplasia, with small focus of adenocarcinoma was diagnosed in one patient. Minimal changes were noted in the myometrial tissue. Leiyomyomata were present in 19 patients, with either intramural location ($n = 15$) or both intramural and subserosal location ($n = 4$). Adenomyosis was observed in 4.5% of patients.

Cervical histology

Minimal changes in cervical histology were observed. Twenty-four simple hyperplasia of the endocervical mucosa were noted. Cervical dysplasia (CIN 1) was present in one case. The exocervix revealed no histological abnormalities.

Breast histology

Of the 100 breast pathological examinations achieved, a marked reduction of glandular tissue and a proliferation of fibrous connective tissue were observed in 93%. Ducts and involuted lobuloalveolar structures were embedded in a dense, hyalinized fibrous tissue. Severe lobular atrophy was observed in 7% of the cases, with mildly atrophic or stromal changes noted in 86% and 7%, respectively. Fibrocystic lesions were reported in 34 cases and two adenofibromas were described. None of the samples showed atypical hyperplasia, in-situ breast carcinomas or features of gynaecomastia. No intraductal papilloma or papillomatosis were observed.

Discussion

Ovarian ageing is a lengthy, complex process characterized by the quantitative and qualitative attrition of ovarian follicles. From their early thirties to their early forties, women are expected to exhaust three-quarters of their follicular reserve (Block, 1952), a phenomenon that exerts a determining effect on their natural as well as medically assisted fertility potential (Gougeon, 1996; Reuss et al., 1996). Low response to ovarian stimulation frequently reflects an age-related decline in reproductive performance, but the same phenomenon may occur in young patients (Fasouliotis et al., 2000; Tarlatzis et al., 2003). Some of the latter group of women have so-called 'occult ovarian failure' shown by elevated FSH serum concentrations (Cameron et al., 1988), but others have normal serum FSH and no apparent reason for repeated low responses to aggressive stimulation protocols (Fasouliotis et al., 2000; Tarlatzis et al., 2003).

Androgens have been shown, in animal models, to play major roles in the process of folliculogenesis, namely the promotion of follicular recruitment (Billig et al., 1993; Hillier and Ross, 1979) and a positive effect on follicular

proliferation (Vendola et al., 1998; Weil et al., 1998). Moreover, androgens are likely to impact folliculogenesis in monkeys by increasing the number of FSH receptors expressed in granulosa cells (Weil et al., 1999). Besides these experimental data, further evidence for a potential positive effect of androgens on follicular proliferation and growth in humans emerged from pharmacological or pathological models. Firstly, a long-term exposure to large doses of exogenous testosterone in FTM transsexuals has been associated with morphological features of polycystic ovary syndrome (PCOS), with a significant increase in the number of small antral follicles (Spinder et al., 1989). Secondly, polycystic-like ovaries have been described in women with non-ovarian causes of hyperandrogenism, such as congenital adrenal hyperplasia and androgen-producing tumours. Lastly, high androgen production may account for the antral follicle excess usually observed in patients with PCOS (de Leo et al., 1998), as suggested by a highly significant positive correlation between the number of small follicles and androgen concentrations (Jonard et al., 2003; Pigny et al., 2003). Altogether, these data suggest that, in non-human primates and in human beings, androgens play a critical role in the control of follicular development and in follicular sensitivity to FSH. Hence, many authors assume that low doses of androgen supplementation during the early phase of follicular recruitment might improve the number of small antral follicles as well as improve the ovarian sensibility to FSH. However, regarding the length of the folliculogenesis process, it is conceivable that androgen therapy should be given for several months in order to be of benefit. This emerging concept requires precise knowledge of the effects of long-term androgen therapy, especially on the female genital tract.

FTM transsexual patients represent a particularly interesting model to study these effects, even though there are differences in the dosage of androgen administration used in this population as compared with the low testosterone supplementation proposed in infertile women. Many studies, with limited numbers of patients, reported ovarian changes after chronic androgen exposure (Amirikia et al., 1986; Miller et al., 1986; Pache et al., 1991; Spinder et al., 1989). These studies, in line with experimental data in an androgenized monkey model (Vendola et al., 1998) and with pathological in-vivo models, such as congenital patients with adrenal hyperplasia or virilizing tumours (Kase et al., 1963), reported macroscopic and microscopic ovary aspects very close to the one observed in ovaries from women with idiopathic PCOS. The present series revealed, among the 112 FTM transsexual patients exposed to high doses of exogenous androgens, a 79.5% prevalence of histological aspects of idiopathic PCOS. As previously described (Futterweit and Deligdisch, 1986; Pache et al., 1991), ovarian volume was increased, probably in relation to the high number of antral follicles and stromal hyperplasia. Although the role of sex steroids in preantral follicle development remains unclear, recent studies highlight the effect of androgens in early follicular growth. In cultured mouse preantral follicles, androgen treatment stimulates follicullar growth (Murray et al., 1998). In intact monkeys, androgen treatment, acting through androgen receptors, increases the number of preantral and small antral follicles by up to 1 mm in diameter (Hillier et al., 1997; Vendola et al., 1998; Weil et al., 1998). Androgens promote both theca interna cell and granulosa cell proliferation, and inhibit apoptosis (Takayama et al., 1996; Vendola et al., 1998). This effect on folliculogenesis predominates in small follicles, due probably to their richness in androgen receptors. All these data are consistent with the induced PCOS-like ovarian aspects observed in FTM transsexual women exposed to long-term androgenotherapy.

The physiological role of androgens in the uterus is not fully clear. Androgen receptors are detected at all phases of the menstrual cycle in the normal uterus both in the epithelium and in the stroma, with a higher concentration in the latter. Endometrial atrophy of the uterine mucosa was observed in about 50% of patients after chronic androgen exposure, while cervical and myometrial histologies do not differ from those of normal women. Atrophic effect of androgen therapy on endometrium has already been described both in FTM transsexuals and post-menopausal women (Perrone et al., 2009; Zang et al., 2007). However, this study's data revealed proliferative and endometrial hyperplasia in 54 and eight subjects, respectively. These endometrial aspects were neither correlated to patient's age nor to their body mass index. Although the testosterone effects on the human endometrium remain unclear, most in-vitro and in-vivo data are consistent with an absence of stimulating effect on endometrial proliferation (Hickok et al., 1993; Perrone et al., 2009; Rose et al., 1988; Zang et al., 2007). Whereas, pathophysiological mechanisms leading to endometrial hyperplasia during androgen therapy remain debated, increased serum oestrogen concentrations after conversion of androgens and insulin resistance may be involved (Lathi et al., 2005). Overall, the chronic lack of progesterone accompanying anovulation, hyperinsulinaemia and hyperandrogenaemia can lead to a net stimulatory effect on endometrial proliferation and hyperplasia. As hyperandrogenism and hyperinsulinaemia are positively correlated in women with anovulation (Dunaif, 1997), it is difficult to distinguish the specific actions of insulin or androgens on the endometrium.

Prevalence of uterine adenomyosis was 4.5%, which is not significantly different from the prevalence in the general population. Although physiopathological mechanisms involved in the genesis of adenomyosis, remain unclear, androgens might not play a major role in opposition to other sexual steroids such as oestrogens and progesterone. One case of endometrial adenocarcinoma, diagnosed on pathological examination in an asymptomatic woman, was observed. This entity had never been previously described in the literature.

Regarding the breast, four studies reported the findings of androgen effects, with variable pathological findings, between intralobular fibrous stroma, extralobular fibrous stroma and lobular atrophy (Burgess and Shousha, 1993; Sapino et al., 1990; Slagter et al., 2006). Buress and Shousha reported no significant effect of long-term androgen administration on the female breast tissue. This study observed a marked reduction of glandular tissue and a promotion of fibrous connective tissue in 93% of the cases. The variability of stromal connective tissue and epithelial changes in this study's patients following androgen administration may be related to the peripheral interconversion of androgens to oestrogens. Thus, effects of androgenotherapy probably reflected the simultaneous action of androgens and oestrogens. Some authors reported an association between circulating testosterone and the risk of developing breast

cancer in post-menopausal women (Key et al., 2002; Missmer et al., 2004). In addition, epithelial hyperplasia has been shown to be associated with increased androgen concentrations (Secreto et al., 1983), but this study found no significant increase in the incidence of epithelial hyperplasia or breast carcinoma. These data are consistent with the absence of evidence of increased breast cancer risk in women displaying hyperandrogenism, such as PCO patients or women affected by virilizing tumours. Thus, androgen therapy given to FTM transsexual patients does not seem to increase the chances of premalignant or malignant-associated changes in the breast tissue.

In conclusion, this retrospective report presents the largest series of histopathological examinations of the genital tract in FTM transsexual patients, after at least 6 months of androgen therapy. Long-term androgen exposure often leads to changes in ovarian architecture, with macroscopic and microscopic ovary aspects resembling the one observed in ovaries from women with idiopathic PCOS. Despite these ovarian modifications, uterine histology does not seem to be very different as compared with non-hyperandrogenic women. Regarding the breast, a marked reduction of glandular tissue and a promotion of fibrous connective tissue were noted, without epithelial aspects of hyperplasia or breast carcinoma. This is consistent with the previous data that concluded that androgens had a non-premalignant or malignant effect on the breast tissue.

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